Western Blot Transfer Buffer (Rapid, Ice-Free, Powder)

Introduction

Western blot transfer buffer is a core reagent used in Western blot experiments to transfer proteins from polyacrylamide gels to solid-phase membranes (such as PVDF or nitrocellulose membranes). Its working principle is based on electroblotting technology: under the influence of an electric field, negatively charged proteins migrate out of the gel and are adsorbed onto the surface of the solid-phase membrane, forming a "blot" that faithfully reflects the protein distribution pattern within the gel.

This product is an ice-free rapid-type transfer buffer, compatible with Tris-Glycine and Bis-Tris transfer buffer systems. Through a proprietary formulation, it significantly reduces heat generation during wet transfer, enabling efficient transfer without the need for an ice bath. Distinguished from standard transfer buffers (transfer time 60–120 min, 200–300 mA, ice-bath required) and rapid transfer buffers (transfer time 20–25 min, 400 mA, ice-bath/room temperature), this product enables wet transfer at room temperature in 15–40 min (at 400 mA), with minimal temperature rise during the process and no cooling measures required. It is the most convenient and least demanding model among the three, particularly suitable for laboratories without ice-bath equipment or for high-throughput continuous transfer applications. This product offers broad applicability, with transfer time flexibly adjustable (15–40 min) according to gel concentration. Under recommended conditions (400 mA, 1.0 mm 6% gel), proteins below 150 kDa can be completely transferred, while proteins >150 kDa require only 5–10 min of extended transfer time.

This product is supplied as a ready-to-use powder formulation. Anhydrous ethanol is used as a substitute for methanol during preparation, avoiding the use of toxic reagents. Each pouch prepares 1 L of 1× working solution and remains stable for 12 months when stored at room temperature.

Protocol

1. Preparation of Transfer Buffer (1×)

   a. Transfer the entire contents of one pouch into a clean beaker.

   b. Add approximately 600 mL of deionized water or double-distilled water and stir to dissolve.

   c. Bring the volume to 800 mL with additional water and mix thoroughly.

   d. Immediately before use, add 200 mL of anhydrous ethanol and mix well to obtain 1 L of 1× transfer buffer.

***Note:** Analytical grade or higher purity ethanol is recommended.

   e. Adjustment (optional): For target proteins with high molecular weight (>150 kDa) or strong hydrophobicity,

SDS may be supplemented to a final concentration of 0.025–0.1% to enhance transfer efficiency.

2. Transfer Procedure (Wet Transfer)

a. Equilibration: After electrophoresis, equilibrate the gel in transfer buffer for 5 min × 3 times. PVDF membranes should be pre-wetted with methanol for 15–30 seconds, rinsed with deionized water, and then equilibrated in transfer buffer for 5 min; NC membranes can be equilibrated directly in transfer buffer.

b. Assembly of the transfer "sandwich" (from anode to cathode):

(+) Anode (red plate)

- Filter paper (2–3 layers, pre-soaked in transfer buffer)
- PVDF membrane (equilibrated)
- Gel (equilibrated)
- Filter paper (2–3 layers, pre-soaked in transfer buffer)

(–) Cathode (black plate)

*Note: After placing each layer, gently roll with a glass rod or roller to thoroughly remove any trapped air bubbles between layers, as bubbles can cause localized transfer failure. The gel should face the cathode (black side) and the membrane should face the anode (red side).

c. Transfer: Transfer the assembled "sandwich" to the electrotransfer tank and add transfer buffer to completely immerse the assembly. Transfer is performed at constant current 400 mA (ice bath not required). For 1.0 mm PAGE gels, proteins below 150 kDa can be completely transferred using the durations specified in the table below:

Gel Concentration	Recommended Transfer Time
6%	15 min
8%	20 min
10%	30 min
12%	35 min
15%	40 min
4-15%	40 min

***Note:**

1. For proteins >150 kDa, extend transfer time by 5–10 min as needed.
2. For 1.5 mm gels at any of the above concentrations, extend the transfer time by 5–10 min accordingly.

d. Post-transfer processing: Remove the membrane. Ponceau S staining may be used to preliminarily confirm transfer efficiency before proceeding to blocking and subsequent antibody incubation steps.

Note

1. Storage and shipping conditions: Store at room temperature; stable for 12 months. Ship at room temperature.

2. This product is compatible with Tris-Glycine and Bis-Tris transfer buffer systems.

3. This product is for scientific research use only.

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